Environmental Health Perspectives
Vol. 41, pp. 189-193, 1981

Mutagenicity Studies of Vinyl Chloride

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Mutagenicity studies in both man and in test organisms clearly demonstrate positive mutagenic activity of vinyl chloride. In terms of the mutagenicity studies using a variety of *in vitro* procedures covering both eukaryotes and prokaryotes, positive effects were found. Cytogenetic *in vivo* studies in animals and in humans indicate not only somatic mutations, but also germinal effects with this chemical.

The importance of mutagenicity studies for the evaluation of the genotoxicity of a chemical lies principally in the transmission of that genetic mutation to the offspring. Therefore, in order to evaluate the reproductive hazards to man of a potential carcinogen or mutagen, one must look at the results of mutagenicity testing in short-term tests. Today, a number of test systems are available in different organisms including bacteria, Neurospora, Drosophila, mammalian cells in culture and *in vivo* tests in rodents. Since a number of agents, including viruses and chemicals, can cause heritable mutations, it is important to identify these agents and to understand the different kinds of genetic diseases which they cause.

Table 1. Genetic disease and incidence of anomalies per million live births.

Disease classification	Current incidence per 10^6 live births
Dominant traits	10,000
X-chromosome	400
Recessive traits	1,500
Chromosomal anomalies	
Unbalanced rearrangements	1,000
Aneuploidy	4,000
Congenital anomalies	15,000
Anomalies expressed later	10,000
Constitutional and degenerative diseases	15,000
Total	56,000

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The kinds of genetic disease and the incidence of these anomalies per million live births are listed in Table 1. The most frequent of these diseases concern congenital anomalies and constitutional and degenerative diseases which occur at a frequency of 15,000 per million births. Chromosomal rearrangements, such as unbalanced translocations, occur at a rate of 1 per 1000 while dominant inheritance occurs at 1 per 100. In all, a total of approximately 5.6 per 100 neonates are affected by some kind of genetic anomaly. This constitutes a very large number of children and certainly represents a significant portion of the population (approximately 1/20 births). It is even more surprising to consider that many of the genetically unfit fetuses simply do not survive to term and are aborted spontaneously. It is estimated that as many as one in two conceptuses do not implant or are aborted during early gestation (*1*, *2*), many of these occurring so early that the mother is not even aware of the pregnancy. In fact, even though the overall rate of chromosomal anomalies among live births is 1 in 20, the actual rate of genetic anomalies is probably tenfold higher, and, in this context the role of environmental chemicals which act as mutagens becomes extremely important.

A significant decrease in infant mortality rates results from the control of infectious diseases seen during the past years (from approximately 30% in 1915 to 3% in 1965) (Fig. 1). The contribution of genetic anomalies to infant mortality, however, has increased during the same period from 5% in 1915 to 15% in 1965 (Fig. 1). An environmental component may be involved in the present incidence of birth defects and may be responsible for the increase in genetic aberrations.

With the advance of medical care of the newborn, individuals with genetic abnormalities that would have normally expired at birth are now surviving. An excellent example of this is the individual with Down's Syndrome (trisomy 21) who a decade ago would not have been expected to live much past the age of 12. Today, however, due to the advances in the care of the newborn and in the control of infectious diseases, children with Down's Syndrome may survive well into their forties, and some of the females have even reproduced. One can, therefore, conclude that natural selection has been somewhat neutralized in the past few years. This factor is coupled with the increasing number of both environmental and industrial chemicals which have been only recently identified that can induce genetic damage. Thus, the utilization of natural selection, as well as the increasing amount of chemical exposure, may both be viewed as important factors leading to the increase in genetic abnormalities (congenital anomalies) which have been observed in man in recent years.

An important social measure of the consequences of birth defects is indicated by life years lost, and as seen in Figure 2, this is much greater than that attributable to cancer, stroke or heart disease (*2*).

We can, therefore, ask: what are the consequences of mutations in germ cells? These include a number of events including an increase in spontaneous abortions, infant mortality, and congenital and developmental birth defects. Developmental defects may appear as children with abnormal puberty, neurological disease, higher mortality or as inborn errors of metabolism.

A measure of these consequences obtained from the National Institute of General Medical Science is that 33% of all admissions to hospital pediatric wards are directly related to genetic defects. It has been estimated that each apparently normal individual carries 5 to 8 deleterious genes (possibly lethal) which are heritable and that each couple stands a 3% risk of bearing a genetically defective child. Furthermore, it has been suggested that approximately 80% of the clinical mental retardation in the country arises from genetic causes, and that many types of genetic damage resulting in brain dysfunction go undetected. We also see that 35% of all spontaneous abortions (more than 100,000 cases per year) have a gross chromosomal defect, many of these due to environmental causes.

The types of point mutations which can be induced by mutagens include transitions (in which an AT pair may be replaced by a CG pair), transversions (in which AT may be replaced by TA), insertions (a GC pair may be inserted) or by deletions (AT pair may be deleted). These events are seen in Figure 3, which illustrates some possible genetic effects of radiation or chemical mutagens (i.e., vinyl chloride). Changes in the nucleotide sequence of the DNA molucule may occur and subsequently affect single base pairs, causing transversions or even deletions. In some instances, where long segments of DNA can be affected, inversions, deletions or translocations may result.

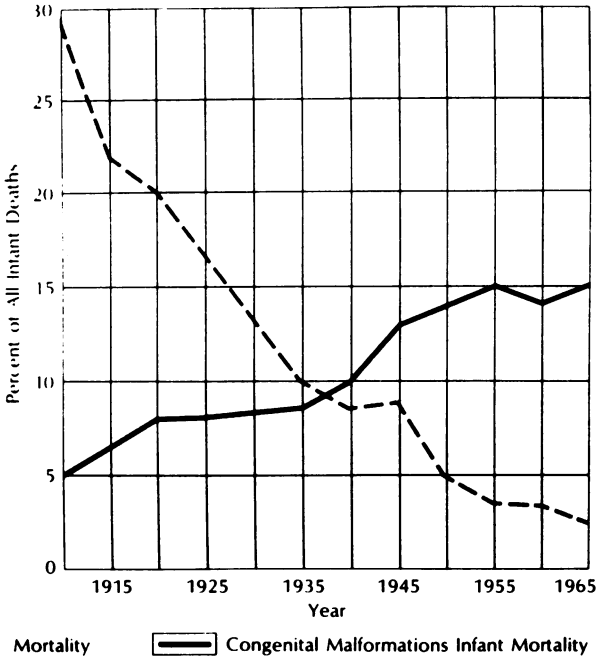

FIGURE 1. Infant mortality rates due (---) to infectious diseases and (——) to congenital malformations.

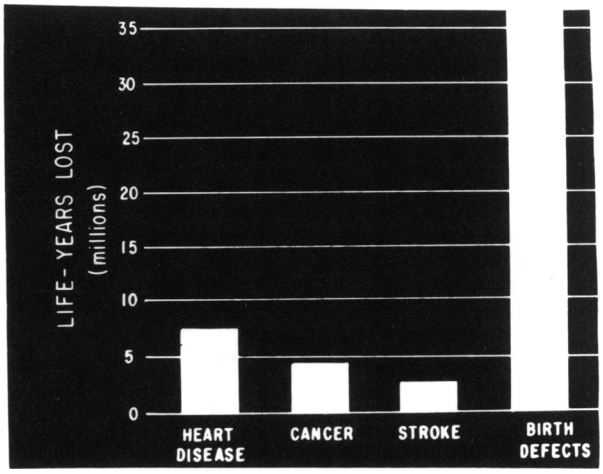

FIGURE 2. Life years lost due to heart disease, cancer, stroke and birth defects.

In addition, triplets can be affected, causing deletions or insertions of bases, all of which may lead to mutations which may be heritable if occurring in germ cells or nonheritable if occurring in somatic tissue. The large number of short-term tests for mutagenicity presently provides a diversity of assays for the testing of these possible genetic lesions. Furthermore, these tests provide the capability for reproducibility of test results and for quantitative measurements.

The simplest of the short term tests for mutagenicity is the Salmonella/mammalian microsome assay, also called the Ames test. The direct mutagenic ability of vinyl chloride in strain TA1530 is seen in Table 2. With a concentration of vinyl chloride as low as 2%, an increase in revertant colonies was observed when compared to the nontreated controls (7 versus 33). In an atmosphere of 20% vinyl chloride, over 100 colonies were observed, while over 200 colonies were observed in an atmosphere of 2% following S9 activation. However, an increase to 500 colonies was observed in plates which were pretreated with Aroclor 1254 and S9-activated (as compared to a spontaneous rate of 12) (Table 3).

A listing of the different test systems as well as the mutagenic activity of vinyl chloride is seen in Table 4. Positive results were reported for vinyl chloride in three bacterial systems. McCann et al. (*4*), Bargin et al. (*5*), Rannug et al. (*6*), and Barstch et al. (*7*), all reported an increase in total number of revertant colonies in Salmonella similar to that seen in Table 2. Greim et al. (*8*) in 1975 reported a positive response in *E. coli*, and Shahin and coworkers (*10*) as well as Loprieno (*11*) showed mutagenicity in yeast both in the gene conversion and in the gene mutation assays.

Similarly, Vogel and Sobels (*13*) as well as Magnusson and Ramel (*14*) reported positive results in the recessive lethal assay in Drosophila. In the Magnusson study, Drosophila were exposed to varying concentrations of vinyl chloride ranging from 1 to 20%. Increased frequencies of both complete and mosaic recessive lethals were seen. They also showed that following pretreatment of phenobarbital for 24 hr an even greater mutagenic effect from vinyl chloride was observed, which indicated a mixed function oxygenase system can be induced in flies which is similar to that reported in mammals.

A number of mammalian cell studies have been completed during the past few years. In one of these studies, increased metabolites were observed in Chinese hamster V79 cells (*15*). However, in a study by Anderson et al. (*16*), an increase in murine embryonic mortality was not seen in females mated to male mice who had received vinyl chloride prior to mating. These authors did report decreased fertility in the males which had received the highest

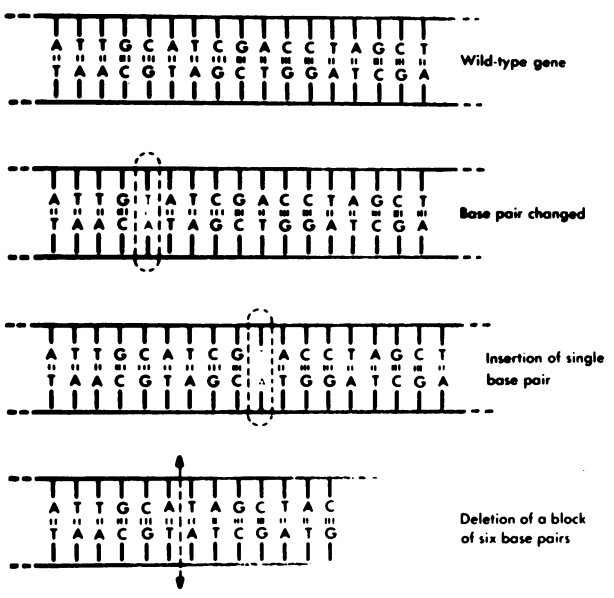

FIGURE 3. Some possible effects of radiation or chemical mutagens.

Table 2. Direct mutagenic activity of VCM towards TA 1530.

VCM concn in atmosphere, %	No. histidine revertants per plate[a]	Spontaneous revertant rate
2	33 ± 6	7
7	48 ± 5	10
12	54 ± 9	7
20	101 ± 16	10

[a]Mean values ± SEM of two assays performed in triplicate.

Table 3. Mutagenicity of VCM towards TA 1530 as observed in simultaneously incubated plates containing either bacteria alone or bacteria and the S9 mix obtained either from control mice or from mice pretreated with Aroclor 1254; VCM concentration in the atmosphere, 2%.[a]

	Control		Aroclor 1254 pretreatment	
	With S9 fraction	Without S9 fraction	With S9 fraction	Without S9 fraction
Number of histidine revertants per plate[b]	206 ± 18	52 ± 9	562 ± 71	102 ± 8
Spontaneous reversion rate	14	16	12	15

[a]Data of deMeester et al. (*3*).
[b]Mean values ± SEM of two assays performed in triplicate.

Table 4. Mutagenicity of vinyl chloride in different test systems.

Test system	Mutagenic activity	References
Salmonella typhimurium (strain TA 1530)	+	McCann et al. (*4*)
	+	Barbin et al. (*5*)
	+	Rannug et al. (*6*)
	+	Bartsch et al. (*7*)
E. coli (K12)	+	Greim et al. (*8*)
Lambda prophage induction	–	Speck et al. (*9*)
Saccharomyces cerevisiae		
Gene conversion/mutation	+	Shahin et al. (*10*)
Gene conversion/mutation	+	Loprieno et al. (*11*)
Neurospora crassa	–	Drozdowicz and Huang, (*12*)
Drosophila recessive lethal assay	+	Vogel and Sobels (*13*)
	+	Magnusson and Ramel (*14*)
Mammalian cell systems		
Chinese hamster V79 cells	+	Drevon et al. (*15*)
Dominant lethal (mice)	–	Anderson et al. (*16*)
Dominant lethal (rats)	–	Short et al. (*17*)
Human studies		
Chromosomal aberrations in workers	+	Ducatman et al. (*18*)
	+	Fumes-Cravioto et al. (*19*)
Chronic lymphocyte cultures	+	Kucerova (*20*)
	+	Szentesi et al. (*21*)
	+	Hansteen et al. (*22*)
	+	Purchase et al. (*23*)
Acute lymphocyte cultures	–	Hansteen et al. (*22*)
Excessive miscarriages in wives of male workers	+	Infante et al. (*24*)

dosage of vinyl chloride, or 50 ppm for 6 hr/day for 5 days. Surprisingly, however, these authors did not regard this fertility decline as "proven" even though 96% of the controls successfully bred while only 55% of the treated animals proved to be successful.

In recent years, chromosomal morphology from cultured peripheral lymphocytes has been studied by a number of investigators in workers exposed to vinyl chloride. In these reports (*18-23*, *25*), increased frequencies of both chromatid and chromosomal damage have been reported. These increases are found to be correlated with the length of exposure as well as with a history of exposure to excursion levels of vinyl chloride during the year prior to sampling (*23*). This study showed 3.18% abnormal cells in the autoclave workers while only 1.08% of the cells sampled from the controls had aberrations. A total of 57 workers were studied.

Ducatman (*18*), as well as Funes-Cravioto (*19*) and their co-workers, reported a similar increase in chromosomal aberrations in 20 workers exposed to vinyl chloride. The most common type of cytogenetic damage reported in these studies were breaks and gaps (*22*), although all types of chromosomal and chromatid danage were seen. Similar results were also reported by Szentesi (*21*), Kucerova (*20*) and Heath and co-workers (*25*). A review of birth defects, and fetal wastage caused by vinyl chloride prepared by Downs et al. (*26*) for The Society of Plastic Industries, Inc. questioned the validity of some of the positive reports with human subjects. Although some of these studies can individually be questioned in terms of experimental design and conclusions drawn, the preponderance of evidence taken as a whole clearly indicate the mutagenic activity of vinyl chloride.

In an important follow-up study on vinyl chloride workers who had initially showed increased frequencies of chromosomal aberrations and who were later removed from that site in the plant to a less exposed area when re-examined two to two-and-one-half years later, showed no differences in chromosomal aberration frequency when compared to controls either in the first study or in the follow-up study (*22*). The original breakage rates of these workers were approximately 3.4%, but upon retesting, only 1.9% of the cells examined were found to have aberrations. These data are extremely important, in that they clearly demonstrate increased cytogenetic aberrations in vinyl chloride workers as well as provide documentation for a relationship between a reduction in exposure to vinyl chloride and normalized chromosomal breakage frequency.

Although the majority of mutagenicity studies in vinyl chloride have been found to be positive, there are, nonetheless, others which show no response (Table 3). These include one study using two different strains of Neurospora (*12*) a lambda prophage induction test (*9*), and dominant lethal tests in both mice (*16*) and rats (*17*). In addition, a cytogenetic study in man was also found to be negative in which both sister chromatid exchange frequencies, as well as chromosomal breakage, were studied following one five minute acute exposure to vinyl chloride (*22*). However, the few studies which show no effect are certainly not persuasive in light of the volume and diversity of the other assays in which positive results are seen.

In man, there are also some epidemiological data which document an increase in spontaneous abortions in the wives of workers exposed to vinyl chloride (*24*). Only husbands (workers) were interviewed in this study, and results showed increased spontaneous abortions in the wives of workers. However, had this report interviewed the wives of the workers, the percent of the observed frequency of spontaneous abortions would have been even more dramatic; therefore the results of this study should be considered conservative.

In summary, there are now sufficient data from mutagenicity studies in man and in test organisms to clearly demonstrate positive mutagenic activity of vinyl chloride. In humans, there are data to indicate not only somatic mutations, which are seen as increased frequencies of chromosomal aberrations in lymphocytes of workers, but also increased spontaneous abortions in the wives of vinyl chloride workers, probably due to mutations occurring in the germ cells of the husbands. There are probably an even greater number of spontaneous abortions which had occurred in these people than those reported and which were simply not detected.

Thus, the hazard of vinyl chloride in reproductive studies is indeed considerable. There is a need for more epidemiological studies to determine the extent of the danger, but that certainly does not detract from the immediate need for an assessment and for public health intervention for exposure of both males and females to vinyl chloride and to structural analogs, using vinyl chloride as a biological model.

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